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Effects of Oestradiol-17β and Progesterone on Total and Nuclear-Protein Synthesis in Epithelial and Stromal Tissues of the Mouse Uterus, and of Progesterone on the Ability of these Tissues to Bind Oestradiol-17β

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1. A method is described for separating uterine epithelium that is 80% pure and connective-tissue stroma that is 60% pure. This was used to study the effects of steroid hormones on total and nuclear-protein synthesis in these tissues. 2. Oestradiol-17β given alone produces mitoses in the epithelium but not in the stroma. It stimulated incorporation *in vitro* of [^{14}C]lysine into total protein, histones and acidic nuclear proteins to a greater extent in epithelium than stroma. Incorporation into acidic nuclear proteins was most markedly stimulated, reaching four to six times the normal value 4h after treatment, and then declining rapidly. This peak was only seen in epithelial preparations. 3. After pretreatment with progesterone, oestradiol-17β has the reverse effect, producing mitoses only in stroma. Progesterone alone had no effect on the amounts or rates of incorporation of [^{14}C]lysine into stromal nuclear proteins, but changes after oestradiol-17β treatment were similar to those seen in epithelium with oestradiol-17β alone. In the epithelium, progesterone alone depressed incorporation into histones and acidic nuclear proteins, but did not abolish the subsequent response to oestradiol-17β. With this treatment there was a rapid, large and transient increase in incorporation into epithelial total protein not seen with oestradiol-17β alone. 4. Progesterone had no qualitative effect on the distribution of specific oestrogen-binding proteins, as judged by sucrose-density-gradient centrifugation. However, progesterone treatment increased the uptake *in vivo* of [6,7-^{3}H]oestradiol-17β by stroma, and it is possible that this is important although the differences were not apparent after labelling *in vitro*.

Progesterone alters the response of the mouse uterus to oestradiol-17β. Given alone, oestradiol-17β produces many mitoses in the luminal and glandular epithelia, but none in the connective-tissue stroma. After treatment with progesterone this pattern is reversed, and oestradiol-17β produces many mitoses in the stroma but few in the epithelium (Martin & Finn, 1968).

Among the early responses of the uterus to oestradiol-17β alone is an increased synthesis of nuclear proteins (Teng & Hamilton, 1969). Since it is generally thought that nuclear proteins play an important part in the regulation of cell metabolism, we decided to test whether progesterone pretreatment altered the nuclear-protein synthesis responses of these tissues to oestradiol-17β. In order to study this problem it was first necessary to devise a method of separating uterine stroma and epithelium.

Using this separation technique we also investigated the effects of progesterone on the ability of uterine tissues to bind oestradiol-17β. Specific oestrogen-binding proteins are required for oestrogen action (Gorski, Toft, Shyamala, Smith & Notides, 1968), and we thought it possible that progesterone modifies oestrogen responses by affecting the synthesis or distribution of these proteins.

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METHODS

Animals and their treatment

The mice were the randomly bred albino strain used by Martin & Finn (1968). Ovariectomy was performed through a dorsal incision under tribromoethanol (Avertin; The Bayer Products Co., Surbiton-upon-Thames, Surrey, U.K.) anaesthesia 2 weeks before the start of hormone

treatment. Hormones were injected subcutaneously in 0.05ml of arachis oil. All the mice were primed with 0.1μg of oestradiol-17β on 2 consecutive days. This produced uteri of higher and more uniform weight than those obtainable from unprimed animals. Starting 2 days after priming, half the animals in each experiment were given 1mg of progesterone daily for 3 days. Oestradiol-17β (0.05μg) was given on the sixth day after priming and the animals were killed at various times thereafter.

Fractionation of uteri

Initially we attempted to separate whole endometrium (stroma and epithelium) from myometrium by scraping with various instruments. Under a dissecting microscope it seemed that the whole endometrium was removed cleanly, leaving a smooth-surfaced tough layer that we thought was myometrium. However, histological sections revealed that only the epithelium had been removed, leaving the stroma intact. Subsequently we found that the epithelium could easily be removed by gently shearing the individual uterine horns between the pestle and mortar of a Potter–Elvehjem homogenizer containing 0.25M-sucrose–3mM-$CaCl_2$. This left the rest of the uterus apparently intact (Plate 1*a*). The homogenizer used had a clearance of 150μm. The epithelia from several uteri were combined by shearing the individual horns separately in the same 2ml of homogenizing medium.

The de-epitheliated uteri were homogenized in 3ml of 0.25M-sucrose–3mM-$CaCl_2$ for 1min in a Silverson homogenizer (Silverson Machines Ltd., London S.E.1., U.K.) at top speed. The homogenate was filtered through 100-mesh wire gauze to remove large particles that resisted homogenization. Microscopic examination of this residue showed it to be predominantly muscle. The filtered homogenate was presumed to contain most of the stromal material and some muscle (see below).

Isolation of nuclei

Epithelium. The epithelial preparations were homogenized briskly in the Potter–Elvehjem homogenizer to break up the cells, and filtered through 200-mesh wire gauze to remove any large fragments. The homogenate was centrifuged at 300*g* for 10min at 4°C. The crude nuclear pellet was resuspended in 5ml of 2M-sucrose–3mM-$CaCl_2$ and centrifuged at 50000$g_{av.}$ for 30min at 4°C. The supernatant was removed by suction, leaving the nuclear pellet.

Stroma. The stromal homogenate was centrifuged at 2000$g_{av.}$ for 10min at 4°C. This left fatty tissue and some fine fibrous material in suspension. The supernatant was discarded and the pellet suspended in 0.25M-sucrose–3mM-$CaCl_2$. This was filtered through 200-mesh wire gauze and the nuclei were prepared as above.

Nuclei from both fractions stained with Azure A were as a routine examined under the microscope to confirm that they were substantially free of cytoplasmic contamination.

Incorporation in vivo *of* [^{3}H]*thymidine as a check on separation of epithelium and stroma*

Two groups of eight mice treated with oestradiol-17β and progesterone plus oestradiol-17β were injected with 1.5μCi of [^{3}H]thymidine (5Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.)/g body wt. 15h and again 17h after oestradiol-17β treatment. At 19h the animals were killed and a short length of one horn from each animal was fixed in Bouin's fluid for radioautography. Epithelial and stromal nuclei were isolated from the remaining tissue. A drop of each preparation was smeared on a slide, fixed immediately in ethanol–acetic acid (19:1, v/v) and used for radioautography.

After five washes with cold 0.5M-$HClO_4$ the specific radioactivity of the DNA in the nuclear pellets was determined.

Radioautographs of uterine sections and nuclear smears were prepared by using Ilford K-5 liquid nuclear emulsion and exposed at 5°C for 6 weeks. To determine the proportion of labelled nuclei in the nuclear smears, 600 nuclei were counted on each slide. The number of labelled nuclei was expressed as a percentage of the total number counted. The proportions of labelled nuclei in the sections were determined by counting 300 nuclei in the epithelia and 600 in the stroma.

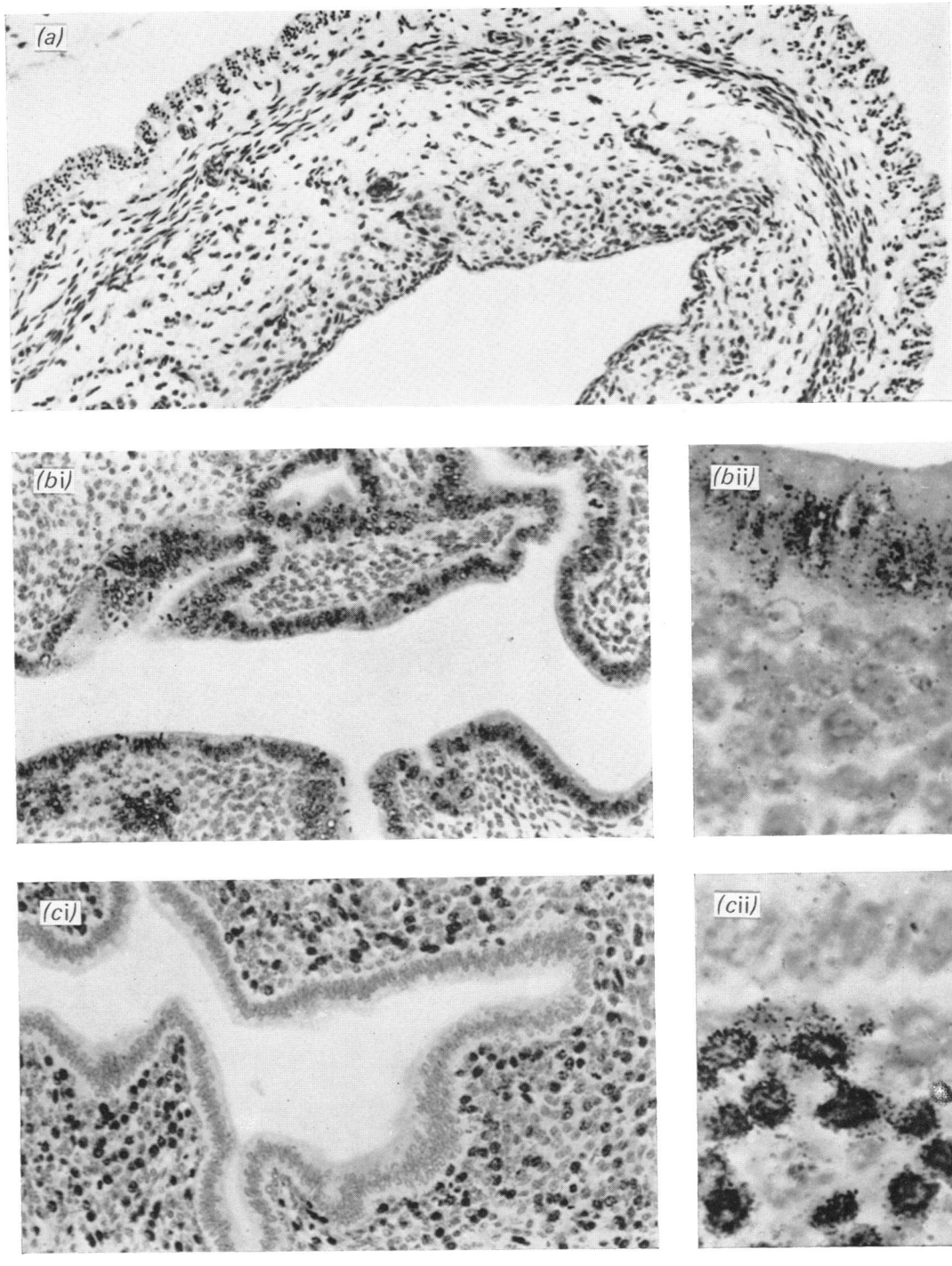
Incorporation in vitro *of* [^{14}C]*lysine*

Uteri from treated animals were dissected out and split longitudinally to expose the luminal surface. Six to eight horns were incubated together for 1h at 37°C in 1.5ml of Krebs–Ringer phosphate buffer (pH7.4) medium 2 (Dawson & Elliott, 1959) containing 1μCi of [^{14}C]lysine (180μCi/mmol; The Radiochemical Centre). After incubation the uteri were removed and stored at −20°C until they could be used. Freezing did not affect the results. Two control incubations were used: (*a*) uteri were incubated in the presence of puromycin (300μg/ml), and (*b*) [^{14}C]lysine was added at the end of the incubation period and the uteri removed 1min later.

Stromal and epithelial preparations were prepared as described above, and 0.5ml of each preparation was taken for the measurement of incorporation of [^{14}C]lysine into total protein. Nuclei were prepared from the remainder.

EXPLANATION OF PLATE 1

The figures show transverse sections (5μm) of uteri fixed in Bouin's fluid and stained with Cole's haematoxylin and eosin. (*a*) Uterus of an untreated ovariectomized mouse after 15s homogenization with a Teflon pestle. The longitudinal and circular muscle and connective-tissue stroma are intact. The epithelium has been removed entirely, leaving a smooth surface to the stroma. (*b*) Uterus of an ovariectomized mouse given 50ng of oestradiol 18h before death. [^{3}H]Thymidine (1μCi/g body wt.) was given intraperitoneally 3h and 1h before death. (i) Low power (×200) and (ii) high power (×1000) views, showing that incorporation of thymidine is restricted to the epithelium. Compare with Fig. 3. (*c*) As for Fig. 2 except that the mouse had received 1mg of progesterone/day for 3 days before administration of oestrogen. (i) Low power (×200) and (ii) high power (×1000) views showing that incorporation of thymidine is restricted to the stroma.


(a)
(bi)
(bii)
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(cii)

Incorporation of [^{14}C]lysine into total proteins

The 0.5ml portions of stromal and epithelial preparations were each treated with 0.5ml of ice-cold 1M-$HClO_4$ to precipitate proteins. The precipitate was collected after centrifugation and washed four times with 2ml of 0.5M-$HClO_4$. The final pellet was dissolved in 1ml of 1M-NaOH. Of this, 0.2ml was used for determination of protein content by the method of Lowry, Rosebrough, Farr & Randall (1951) and 0.5ml was taken for counting of radioactivity in a scintillation counter (Packard model 3375).

Fractionation of nuclear proteins and measurement of [^{14}C]lysine incorporation

The nuclei were suspended in 1ml of ice-cold 0.01M-tris–HCl buffer, pH7.4, containing 3mM-$CaCl_2$, for 10min and then centrifuged at $1000g_{av.}$ for 10min at 4°C. The supernatant, which was decanted, contained globulins and nuclear ribosomes (Frenster, Allfrey & Mirsky, 1960).

Histones were extracted by suspending the nuclei in 1ml of ice-cold 0.2M-HCl for 10min. In some experiments a sample was taken and DNA determined by the method of Burton (1956). The HCl suspension was centrifuged and the supernatant decanted. This contained histones.

The remaining nuclear solids were treated with 1ml of 1M-NaOH for 30min to dissolve the tris- and acid-insoluble proteins. These are referred to as acidic nuclear proteins.

Gel electrophoresis

Some histone fractions were subjected to polyacrylamide-gel electrophoresis by the method of Panyim & Chalkley (1969), for histone separation. A second acid extract of the nuclear residue and acetone precipitate of the tris-soluble material were checked for histones by the same method.

Histone fractions were also run in a 7.5% (w/v) polyacrylamide gel with 0.05M-tris–0.5M-glycine buffer, pH8.4, to determine whether non-histone proteins were present.

Labelling of tissues with [6,7-^{3}H]oestradiol-17β

Labelling in vivo. Mice were injected with 0.1μg of [6,7-^{3}H]oestradiol-17β (235μCi/μg) subcutaneously. The mice were killed 1h later and their uteri removed; four uteri were combined for each analysis.

Labelling in vitro. Uteri were incubated for 30min at 4°C in 5ml of 1.0nM-[6,7-^{3}H]oestradiol-17β in Krebs–Ringer phosphate buffer, pH7.4; 12 uteri were used for each incubation. The uteri were washed three times with 5ml of the Krebs–Ringer phosphate buffer at 4°C. Each wash lasted 3min.

After labelling by either method epithelial and stromal fractions were prepared in 2ml of TEM-buffer (0.01M-tris–1.5mM-EDTA–1mM-β-mercaptoethanol) and centrifuged at $10^5g_{av.}$ for 1h. The nuclear oestrogen receptors were extracted from the pellet with 0.5ml of 0.3M-KCl in TEM-buffer for 15min and the insoluble material was sedimented at $5000g_{av.}$ for 30min at 4°C. The radioactivity in the 10^5g supernatant and TEM-buffer extract was counted.

Sucrose-density-gradient centrifugation

Linear 5–20% (w/v) sucrose gradients were prepared, and the sedimentation profiles of bound oestradiol-17β in the 10^5g supernatants and TEM-buffer extracts of pellets analysed as described previously (King, Gordon & Steggles, 1969). Sedimentation coefficients are quoted according to the system of Jensen, Suzuki, Numata, Smith & De Sombre (1969).

Precipitation with protamine

A 0.4ml portion of extract was mixed with 0.2ml of protamine sulphate (7.5mg/ml in water). After standing at 0°C for 10min the precipitate was sedimented at $1000g_{av.}$ for 5min. The radioactivity in the supernatant and precipitate was measured as specific and non-specific bound oestradiol respectively (Steggles & King, 1970).

RESULTS

Separation of uterine components

As expected, radioautographs of uterine sections from animals treated with oestradiol-17β alone had many of the epithelial cells and virtually none of the stromal cells labelled (Plate 1*b*). The reverse was seen after progesterone pretreatment (Plate 1*c*).

Comparison of the proportion of labelled nuclei in epithelium and stroma *in situ* and in epithelial and stromal preparations showed reasonable agreement (Table 1). The results indicate that our epithelial fractions were 75–85% pure. Stromal preparations were approx. 60% pure. The bulk of the contamination was presumably derived from the myometrium.

The specific radioactivities of DNA from the nuclear preparations were in close agreement with these results (Table 1).

Separation of nuclear proteins

Polyacrylamide-gel electrophoresis showed that the 0.2M-hydrochloric acid extract of nuclei contained all of the groups of histones normally recognized and a few extra, faint, bands, which were comparable with the degradation products of histones demonstrated by Panyim & Chalkley (1969). None of the proteins in this fraction moved from the origin into the 7.5% polyacrylamide gel at pH8.4. No histone bands were found in either the tris-soluble or the acid-insoluble fractions.

Incubation experiments

Preliminary studies showed that the amount of [^{14}C]lysine incorporated into total and nuclear proteins was a linear function of time over 1h. The specific radioactivities of proteins isolated from controls with puromycin added, or with radioactivity added at the end of the incubation, were consistently less than 10% of the values from test incubations.

General comparison of epithelial and stromal preparations

Experiments in which DNA was measured showed that the histone content/mg of DNA was

Table 1. [^{3}H]*Thymidine incorporation in uterine epithelial and stromal cells and nuclei isolated from these tissues*

The results are expressed as the percentage of nuclei showing thymidine incorporation, and the d.p.m./μg of DNA in the nuclear preparations. A total of 600 cells was counted in each nuclear smear. In uterine sections (prepared from the uteri) 300 cells were counted in the epithelium and 600 in the stroma. Duplicate groups of four uteri were used in each treatment group.

	% of labelled nuclei				Sp. radioactivity of DNA (d.p.m./μg)			
	Oestradiol		Progesterone+ oestradiol		Oestradiol		Progesterone+ oestradiol	
	1	2	1	2	1	2	1	2
Epithelium *in situ*	73.0	67.4	2.0	1.1	—	—	—	—
Epithelial nuclear fraction	54.5	50.2	10.5	5.5	226	287	9	5
Stroma *in situ*	0.1	0.1	38.4	38.9	—	—	—	—
Stromal nuclear fraction	1.7	1.7	26.7	23.3	16	13	267	197

Table 2. *Incorporation of* [^{14}C]*lysine into proteins of epithelial and stromal fractions from untreated uteri*

Four uteri were used for each incubation. Rates of incorporation are expressed as d.p.m./mg of protein in 1 h.

	Total protein			Histones			Acidic nuclear proteins		
			Epithelium			Epithelium			Epithelium
Expt. no.	Stroma	Epithelium	Stroma	Stroma	Epithelium	Stroma	Stroma	Epithelium	Stroma
1*	3260	3920	1.38	2690	7200	2.88	2210	13950	6.74
	2420			2270			2080		
	2430	2240	0.89	1910	7790	4.14	1970	10480	5.29
	2510			1830			2010		
2	1640	1790	1.06	620	1150	1.86	1000	1870	1.87
	3370	2540	0.76	1120	2160	1.93	700	1780	2.54
3	4860	2870	0.59	3120	7280	2.33	1250	4050	3.24
	6530	3650	0.56	3060	9240	3.01	1330	3820	2.89

* In Expt. 1 the epithelia from two incubations were combined.

about the same in epithelial and stromal nuclei, but epithelial nuclei contained more acidic nuclear protein/mg of DNA than did stromal nuclei (see Figs. 2 and 4). No clear difference was apparent in the rate of incorporation of amino acid into the total proteins of epithelium and stroma, but incorporation into both histones and acidic nuclear proteins was invariably higher in epithelial nuclei than in stromal nuclei. In untreated uteri there was a two- to six-fold difference in rate of incorporation (Table 2). The incorporation into the uterine proteins varied considerably from experiment to experiment but agreed well within each experiment.

Effects of oestradiol-17β on [^{14}C]*lysine incorporation*

Changes in rate of incorporation into stromal and epithelial total protein, histone and acidic nuclear protein at various times after oestradiol treatment are shown in Fig. 1. The graphs were constructed from the results of three experiments each with two or more incubations of uteri from primed, but otherwise untreated, animals (zero-time controls). To compensate for the inter-experiment variability (see Table 2) each result (d.p.m./mg of protein), including the zero-time controls, has been expressed relative to the mean of the appropriate zero-time controls (= 1).

Stroma. Oestradiol-17β injections resulted in slight increases in incorporation into stromal total protein that were apparent 4–6 h after treatment and persisted up to 18 h. Incorporation into stromal nuclear proteins was also increased, but in acidic nuclear protein the increase was apparently transient.

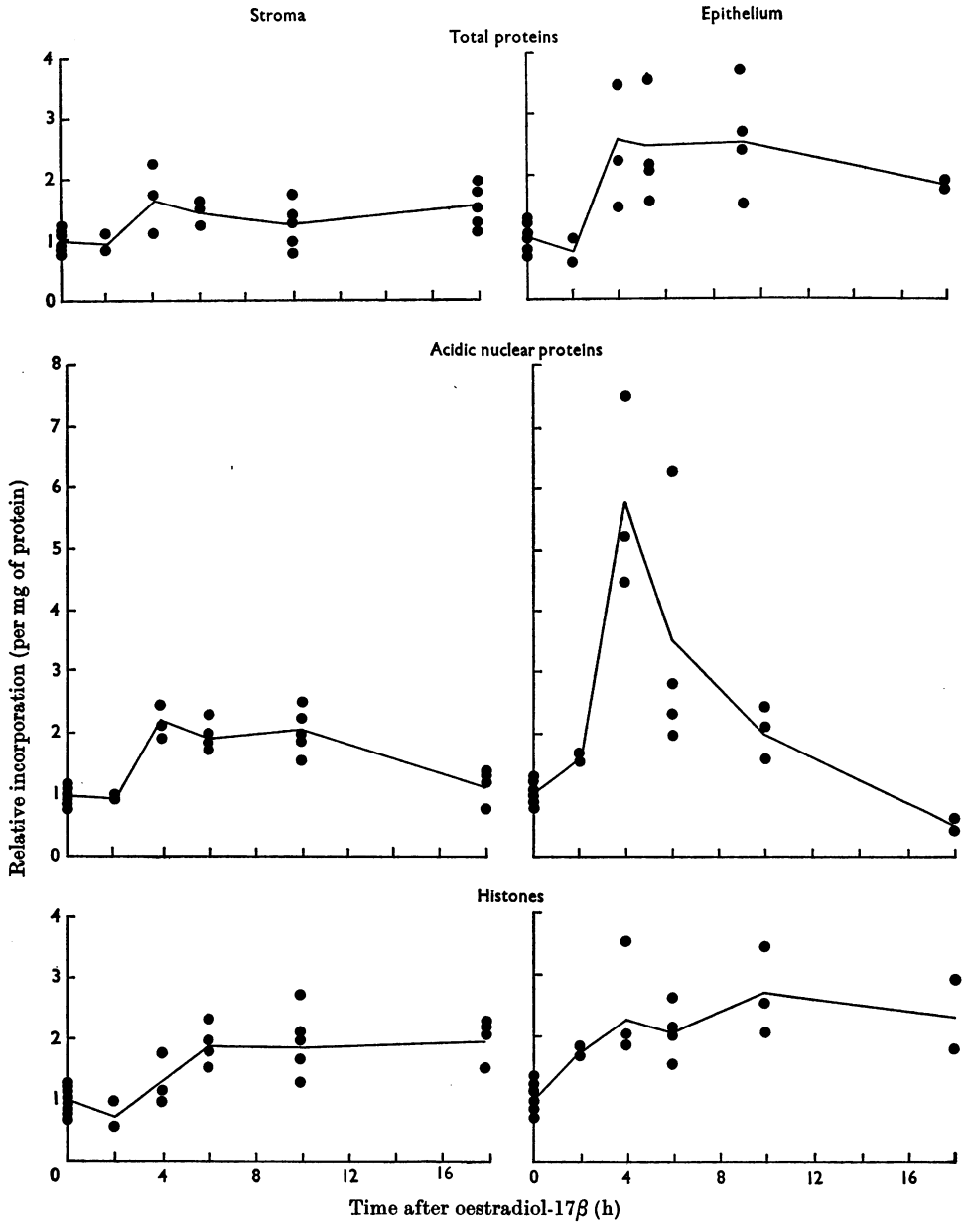

Fig. 1. Effects of oestradiol-17β on incorporation *in vitro* of [^{14}C]lysine into total proteins, acidic nuclear proteins and histones of uterine epithelium and stroma. Uteri were incubated *in vitro* for 1 h, beginning at specified times after hormone treatment *in vivo*. Each point represents the d.p.m./mg of protein for one incubation, relative to the mean value of the zero-time controls incubated at the same time.

Epithelium. Incorporation of [^{14}C]lysine into epithelial total proteins and histones was increased to a greater extent than that into stroma. The epithelial acidic nuclear proteins showed a rapid fivefold increase in rate of incorporation up to 4 h after treatment, followed by a rapid decline to less than zero-time control values by 18 h.

Changes in amounts of nuclear proteins (*Fig.*

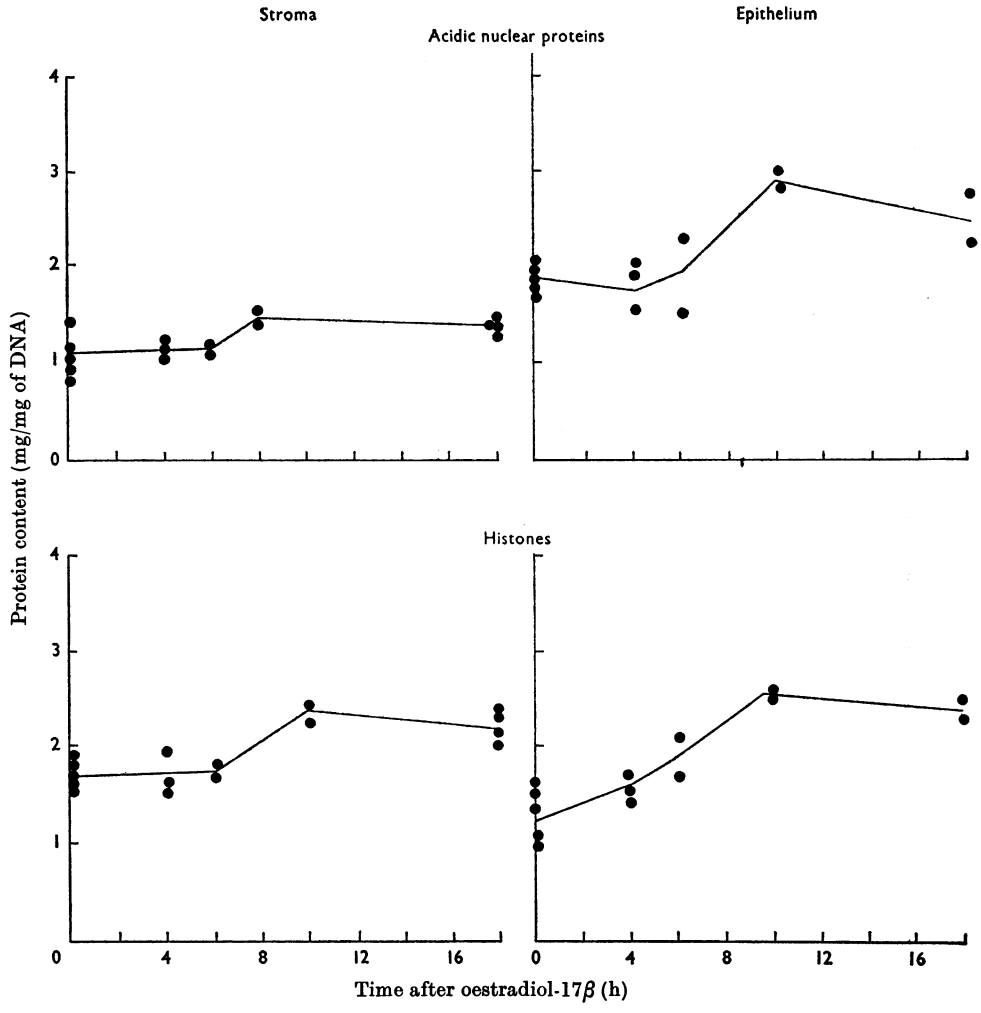

Fig. 2. Effects of oestradiol-17β on the amounts of acidic nuclear proteins and histones/mg of DNA in uterine stroma and epithelium.

2). The amounts of histones and acidic nuclear protein/mg of DNA increased only slightly after oestradiol-17β treatment in the stroma, but quite markedly in the epithelium. These increases were apparent 10h after treatment.

Effects of progesterone

After 3 days of progesterone treatment increased amounts of protein were recovered from stromal preparations. Incorporation of [^{14}C]lysine/mg of protein in each experiment was lower, but the amount incorporated per stromal preparation was slightly increased (Table 3). The amount of histone and acidic nuclear protein/mg of DNA was unaffected (cf. Figs. 2 and 4) and there was no consistent effect on the amount of [^{14}C]lysine incorporated into these proteins (Table 3). Epithelial fractions also contained more total protein but a lower rate of incorporation of [^{14}C]lysine/mg of protein. The amounts of epithelial histone and acidic nuclear protein were unchanged (cf. Figs. 2 and 4), but there was a considerable decrease in the incorporation into both types of protein (Table 3).

Effects of oestradiol-17β after progesterone

Stroma. In these experiments incorporation/mg of total stromal protein was increased considerably 4–10h after oestradiol-17β treatment, but had declined by 18h (Fig. 3). However, by 18h the

Table 3. *Effect of 3 days of progesterone treatment (1 mg/day subcutaneously) on total protein content, and incorporation of [^{14}C]lysine into proteins of stromal and epithelial preparations*

The results for the treated animals are expressed as percentages of untreated controls incubated at the same time.

	Total protein				
Stroma Expt. no.	(mg/ preparation)	(d.p.m./ preparation)	(d.p.m./mg of protein)	Histones (d.p.m./mg)	Acidic nuclear proteins (d.p.m./mg)
1	159	120	78	90	133.0
2	226	125	56	100	84
3	198	116	59	78	85
Epithelium Expt. no.					
1	135	108	82	36	51
2	180	118	69	68	76
3	146	130	61	62	74

amount of protein recovered was increased, so that, per preparation, the rate of incorporation was still elevated. There was a rapid fivefold increase in incorporation into acidic nuclear proteins up to 4h after treatment, followed by a sharp decline to less than zero-time control values by 18h. Histone incorporation increased more slowly up to about 10h after treatment and then declined to control values. The increase was somewhat greater than with oestradiol alone. Increases in the amount of histones and acidic nuclear proteins/mg of DNA were detected (Fig. 4), but the values dropped again by 18h. This decline may be related to increase in DNA content, since the cells are well into DNA synthesis by 18h (cf. [^{3}H]thymidine results).

Epithelium. Although this treatment does not cause cell division in the epithelium the nuclear-protein changes were very similar to those seen in stroma with the same treatment, which stimulates stromal division. Similar changes in the amount of acidic nuclear protein/mg of DNA were also observed, but only a small increase in histone/mg of DNA. A major difference was seen in the incorporation into total protein, which was very high 4h after oestradiol-17β treatment, and declined rapidly thereafter, just like the acidic nuclear proteins.

Effect of progesterone on oestradiol binding in epithelium and stroma

Sucrose density gradients of uteri labelled *in vitro* showed that both epithelium and stroma from untreated and progesterone-treated mice contained a cytoplasmic binding component with a sedimentation coefficient of 8S (Figs. 5 and 6). The 10^5g pellets from stroma and epithelium contained a binding component with the same sedimentation coefficient as that in rat uterine nuclei (4–5S). Although progesterone treatment had no effect on the sedimentation profiles, it increased the uptake *in vivo* of [6,7-^{3}H]oestradiol-17β in both the 10^5g pellet and supernatant of the stroma ($P<0.05$ and <0.01 respectively), but not in either preparation from the epithelium. As judged from the protamine precipitation, progesterone also increased the proportion of specifically bound oestradiol in the stromal supernatant. Insufficient epithelium was available for protamine treatment.

The lack of effect of progesterone on the uptake *in vitro* of [6,7-^{3}H]oestradiol-17β (Table 4) is partly due to the low nuclear binding obtained at 4°C (Gorski *et al.* 1968). This would not explain the results for the supernatant.

DISCUSSION

The method described for separating uterine epithelium and stroma gave nuclei of different types: the epithelial nuclei contained more acidic nuclear proteins/mg of DNA than stromal nuclei, and the incorporation of [^{14}C]lysine into both acidic nuclear proteins and histones was consistently higher in epithelial fractions. Comparison of the distribution of [^{3}H]thymidine in sections and epithelial nuclear preparations showed that 75–80% of the nuclei were of epithelial origin. The stromal preparations were more heavily contaminated, presumably with myometrial nuclei. However, the differences in acidic-nuclear-protein content and amino acid incorporation were too great to be accounted for in terms of myometrial contamination alone. We have no direct evidence to show that the total protein is distributed in the same way as the nuclei, and it is possible that leakage of stromal and myometrial proteins into epithelial preparations occurred. The results suggest that this was not a serious source of error, since clear differences were observed in the changes in rate of amino acid

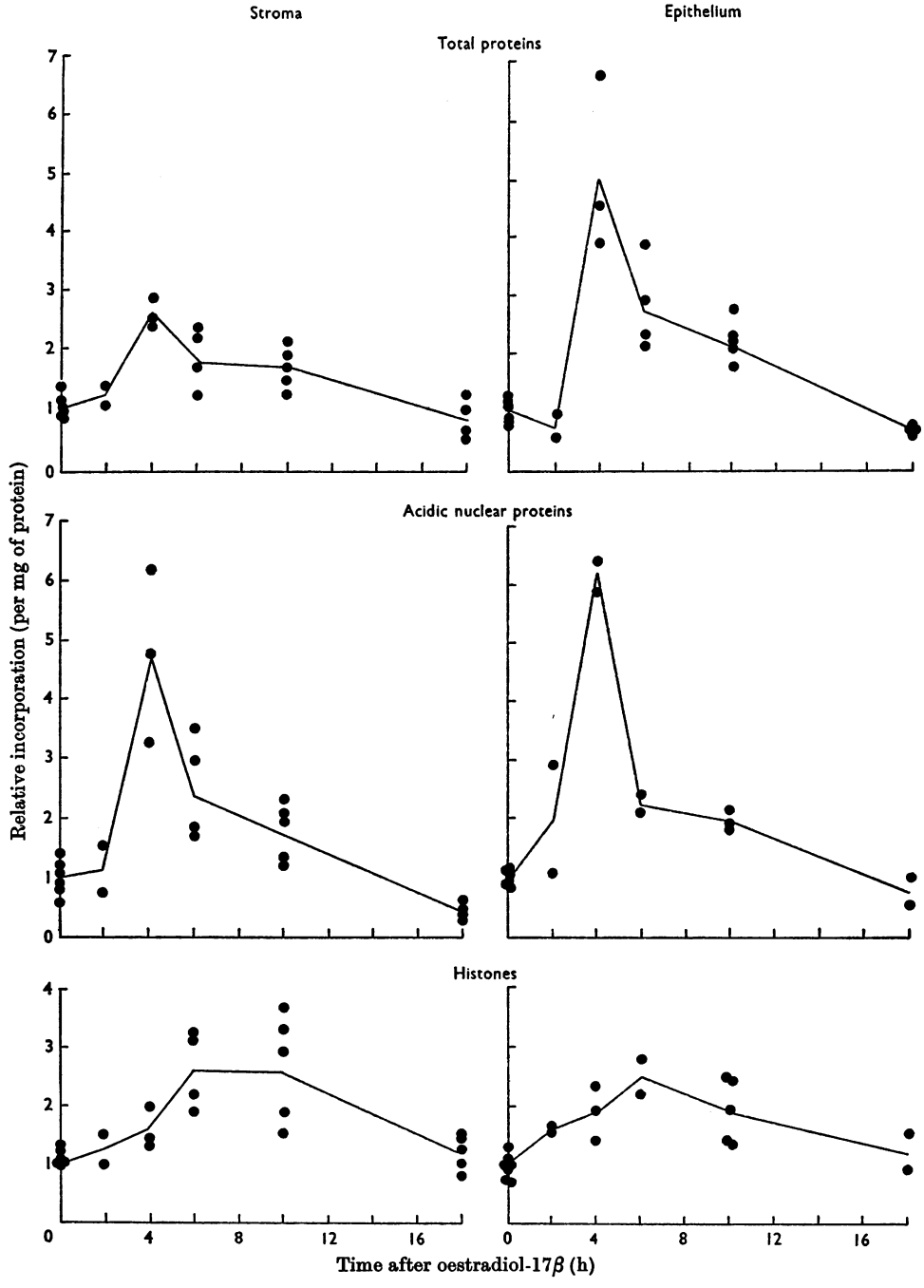

Fig. 3. Effects of oestradiol-17β on incorporation *in vitro* of [^{14}C]lysine into epithelial and stromal proteins after 3 days' pretreatment with progesterone (1 mg/day).

incorporation into total protein after hormone treatment.

The incorporation of [^{14}C]lysine into proteins was almost totally inhibited by puromycin and may be taken to reflect protein synthesis. Although it is not possible to rule out effects due to changes in

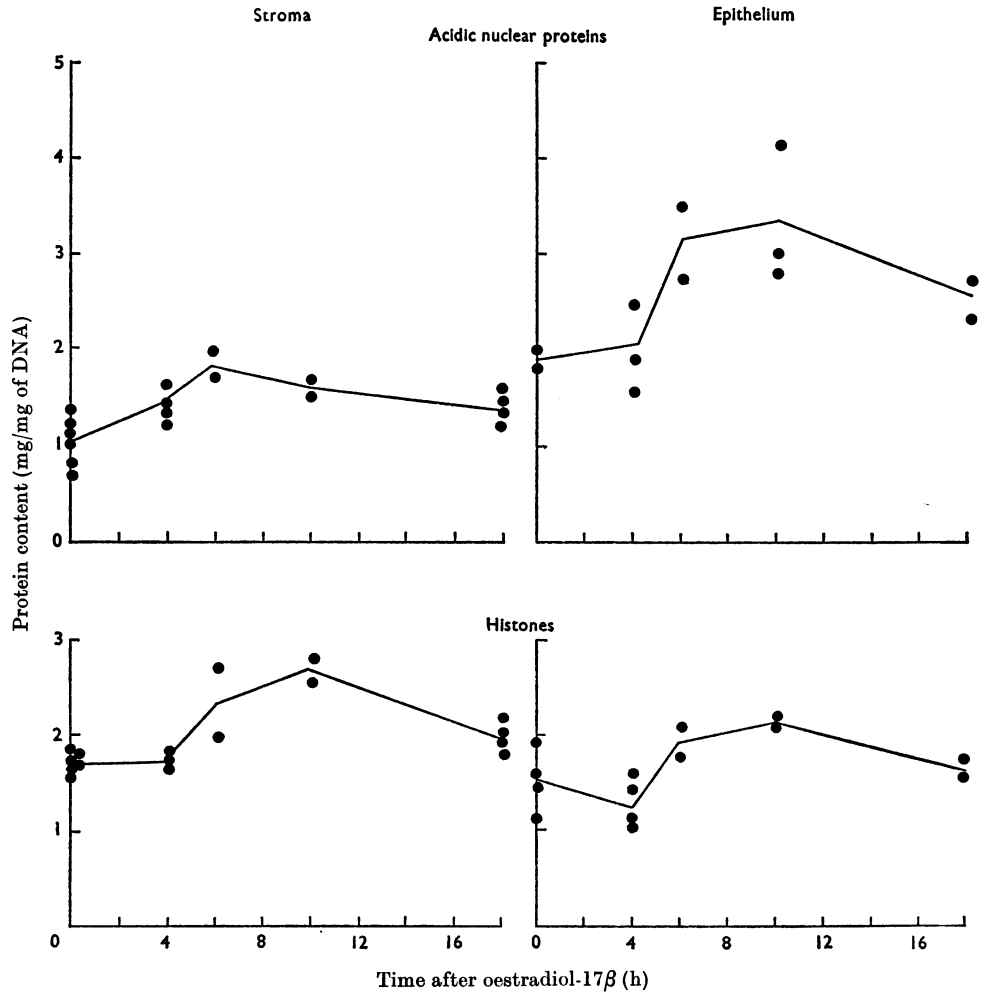

Fig. 4. Effects of oestradiol-17β on acidic nuclear protein and histone content of stroma and epithelium after 3 days' pretreatment with progesterone (1 mg/day).

amino acid pool sizes, the observed changes in protein content make it unlikely that this was a major factor.

The exact function and interrelationship of histones and acidic nuclear proteins are not known. It has been suggested that histones are involved in the masking of genes (Allfrey, Littau & Mirsky, 1964; Wang, 1968), but there is no convincing evidence that these proteins could confer tissue specificity. The acidic nuclear proteins, on the other hand, are thought to act as gene activators, and the work of Paul & Gilmour (1968) suggests that tissue specificity may be conferred by these proteins. From this point of view the difference in acidic-nuclear-protein content of epithelial and stromal nuclei may reflect differences in nuclear function.

There has been considerable debate about the timing of histone synthesis in relation to cell division (Stellwagen & Cole, 1969). Cytochemical studies indicate that histone synthesis occurs only at the same time as DNA synthesis in many slowly dividing tissues (Woodward, Rasch & Swift, 1961; Alfert, 1954; Bloch & Goodman, 1955; Prestcott, 1966). However, Umana, Updike, Randall & Dounce (1964) found that after partial hepatectomy histone synthesis begins before DNA synthesis and continues into S-phase. Incorporation experiments have also shown that histone synthesis can occur independently of DNA synthesis, and even when this is artificially prevented (Flamm & Birnstiel,

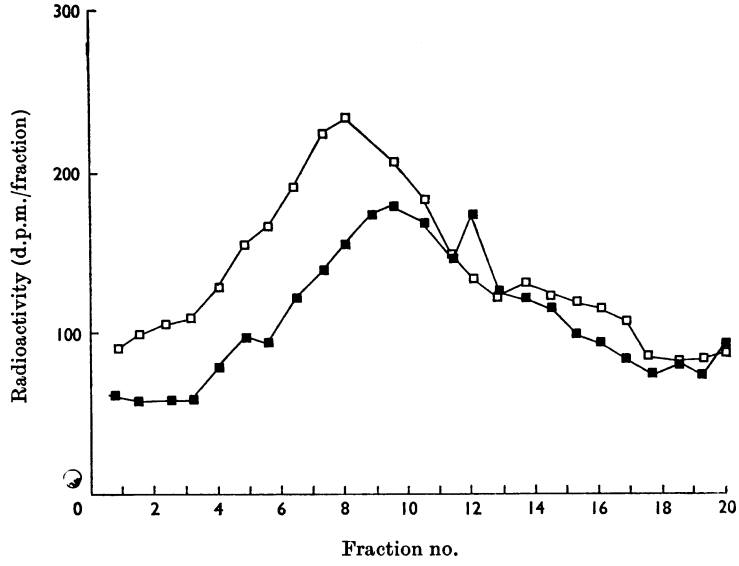

Fig. 5. Sucrose-density-gradient profiles of stromal 10^5g supernatants labelled *in vitro* with [6,7-^{3}H]oestradiol-17β. ■, Untreated; □, progesterone-treated.

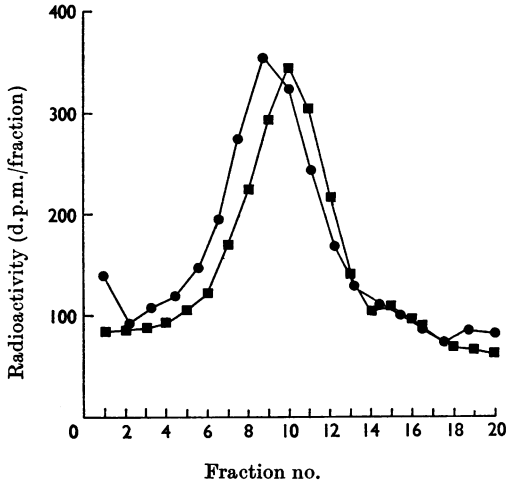

Fig. 6. Sucrose-density-gradient profiles of epithelial 10^5g supernatants labelled *in vitro* with [6,7-^{3}H]oestradiol-17β. ■, Untreated; ●, progesterone-treated.

1964; Sagdopal & Bonner, 1969). The present results support the view that histone synthesis is not necessarily coupled to DNA synthesis. Our results also suggest that histone synthesis precedes DNA synthesis in dividing uterine cells, as in regenerating liver (Umana *et al.* 1964), since [^{3}H]-thymidine incorporation does not increase until 9–10h after oestrogen treatment (Martin & Finn, 1970) whereas histone synthesis was maximal at this time.

Studies on the synthesis of acidic nuclear proteins are few. It seems that these proteins are much less dependent on DNA synthesis than histones, and may be synthesized faster during G_1 than during S phase (Sagdopal & Bonner, 1969). The rapid response of these proteins to hormone stimulation that we have found is in agreement with this.

There is now ample evidence that histones and acidic nuclear proteins continually turn over in non-mitotic tissues (Chalkley & Maurer, 1967, and references cited above). Our results suggest that turnover of both histones and acidic proteins occurs in quiescent uterine tissues, and that turnover rates are fastest in epithelial tissue. The significance of nuclear-protein turnover is not known.

Both stroma and epithelium responded to oestradiol-17β alone by changes in incorporation of amino acids into proteins. The effects were most marked in the epithelium, particularly in the increased incorporation into acidic nuclear proteins. Since the epithelium is the smallest component tissue of the uterus these changes would tend to be obscured when the effects of oestrogens on whole uteri are studied. This effect would be mitigated to some extent by the higher initial rates of epithelial nuclear-protein synthesis. Our results are in broad agreement with those of Teng & Hamilton (1969)

Table 4. *Effect of treatment* in vivo *with progesterone on the binding of* [6,7-^{3}H]*oestradiol-17β in uteri*

Results are expressed as means ±S.E.M. of four determinations.

Method of labelling with [6,7-^{3}H]oestradiol-17β	Amount bound to specified fraction derived from one uterus (d.p.m./uterus)				% of supernatant ^{3}H radioactivity precipitated by protamine (stroma)
	10^5g pellet		Supernatant		
	Epithelium	Stroma ($\times10^{-3}$)	Epithelium ($\times10^{-3}$)	Stroma ($\times10^{-3}$)	
In vivo					
Control	606±179	15.31±1.20	1.66±0.19	1.52±0.35	23±3
Progesterone-treated	986±142	27.10±3.12	1.51±0.32	3.18±0.57	53±7
In vitro					
Control	519±89	1.54±0.22	2.19±0.29	2.28±0.16	86±5
Progesterone-treated	650±32	1.56±0.18	2.07±0.89	2.83±0.18	86±2

for rat uteri pulse-labelled with amino acids *in vivo*. They showed an increase in total nuclear-protein synthesis that was maximal at 8h after treatment, but did not fractionate the proteins further. In our experiments increased amounts of histones and acidic nuclear proteins per mg of DNA were observed. These changes also were greatest in epithelial preparations. Alteration, by progesterone pretreatment, of the ability of stromal cells to respond to oestradiol-17β by division were not accompanied by detectable changes in histone or acidic-nuclear-protein content or turnover. The changes in stromal nuclear-protein synthesis after progesterone and oestradiol-17β treatment were closely similar to those in epithelium after oestradiol-17β alone. However, in progesterone-treated epithelium, which does not divide after oestradiol-17β treatment, comparable changes were observed. Thus in the uterus oestrogen-induced changes in nuclear-protein synthesis cannot be regarded simply as preparative to mitosis.

The possible significance of these changes in non-dividing tissue is not clear. There was a considerable increase in the amount of acidic nuclear protein/mg of DNA, but no great change in histone content. These changes were associated with a very rapid transient increase in total-protein synthesis.

Apart from an increase in total-protein content and depression of epithelial nuclear-protein turnover we were unable to detect any effect of progesterone alone on protein synthesis that would help explain its mechanism of action. Progesterone treatment increased the uptake *in vivo* of [6,7-^{3}H]-oestradiol-17β by stroma, but not that by epithelium.

It is nevertheless unlikely that progesterone has its effects simply by influencing the binding of oestradiol-17β. Oestradiol receptors were present in both stroma and epithelium, yet the effects of progesterone on these two tissues were very different.

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